

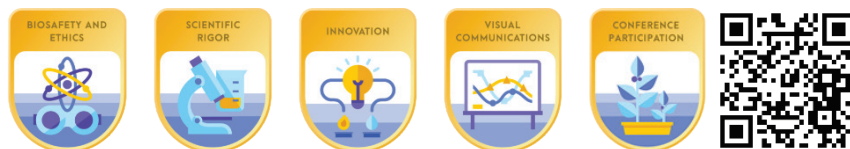
Utilizing an engineered *Escherichia coli* Nissle 1917 as an inflammation-responsive probiotic for celiac disease therapy*

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*Celiac disease is a serious autoimmune disorder that damages the small intestine in response to gluten, a protein found in wheat, rye, and barley. With an estimated 1 in 100 people worldwide affected and the prevalence continuing to rise, there is a growing need for innovative treatments that go beyond strict gluten-free diets. Current therapies rely heavily on dietary restriction, which can be difficult to maintain and do not always prevent inadvertent exposure. This project proposes a synthetically engineered *Escherichia coli* Nissle 1917 (EcN) probiotic designed to reduce gluten-induced inflammation in individuals with celiac disease. The engineered EcN strain detects elevated levels of tumor necrosis factor-alpha (TNF- α), a key pro-inflammatory cytokine upregulated during a gluten-triggered immune response. In response, it secretes interleukin-10 (IL-10), a potent anti-inflammatory cytokine, into the gut environment. By modulating the immune response at the source, this approach aims to reduce intestinal inflammation and improve quality of life for individuals with celiac disease. Incorporating advanced genetic engineering, biosensing, and safety mechanisms, this project explores a novel therapeutic strategy that directly targets the underlying inflammatory pathway of the disease.*

Keywords: Celiac disease, *Escherichiacoli*, inflammation, TNF- α , IL-10



Celiac disease (CD), also known as a gluten sensitive enteropathy, is an autoimmune inflammatory condition triggered by an immune-mediated response to dietary gluten (a protein found in wheat, barley, and rye) in genetically predisposed individuals (Mayo Clinic, 2023). Affecting approximately 1 in 100 people, CD is driven by an inappropriate immune response to gluten peptides, particularly in individuals obtaining the HLA-DQ2 or HLA-DQ8 genetic haplotypes (Posner & Haseeb, 2025). Upon gluten ingestion, the protein is broken down into peptides that the immune system

recognizes, initiating the activation of both innate and adaptive immune responses (Ciccocioppo et al., 2005). Specifically, this response correlates to damage in the small intestinal mucosa—the three-layered membrane that lines the insides of the body and secretes mucus to capture invaders—leading to villous atrophy, gastrointestinal symptoms, malabsorption of important nutrients, and a range of symptoms including abdominal pain, diarrhea, dermatitis herpetiformis, osteoporosis, and neuropathy (Celiac Disease Foundation, 2025).

A distinctive attribute of CD is the

* The author was mentored by Lindsey L'Ecuier from Andover High School. Please direct correspondence to: lindsey.lecuier@andoverma.us. This is an Open Access article, which was copyrighted by the authors and published by BioTreks in 2025. It is distributed under the terms of the Creative Commons Attribution License, which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited.

inflammatory response in the small intestine that is crucial in the progression of the disease (Barone et al., 2022). Inflammation is the body's response to illness, injury, or a foreign object such as germs or toxic chemicals (Cleveland Clinic, 2021). Usually, it is a protective mechanism, but in people with CD, it becomes dysregulated and instead leads to chronic inflammation and tissue damage (Barone et al., 2022). Pro-inflammatory cytokines, in particular tumor necrosis factor-alpha (TNF- α), are key factors in this process (Cleveland Clinic, 2023). TNF- α is produced predominantly by activated macrophages, T cells, and natural killer cells and exists in both soluble and transmembrane forms. It binds to TNFR1 and TNFR2 to trigger signaling cascades related to inflammation, cell survival, and immune cell requirement. TNF- α is a major aspect of many autoimmune diseases, including rheumatoid arthritis, inflammatory bowel disease, and celiac disease, due to its nature to exacerbate immune response, disrupt epithelial integrity, and promote fibroblast activation (Jang et al., 2021). Moreover, elevated TNF- α levels give rise to tissue damage and chronic inflammation in the celiac gut, making it a key therapeutic target in the management of CD (Rezaei-Tavirani et al., 2019).

While current management of CD is solely dependent on lifelong gluten-free diets, adherence is difficult, and cross-contact with gluten-enriched food is common. Cross-contact often occurs through shared kitchen equipment, restaurant meals, mislabeled products, or trace contamination during food process due to the lack of options available in the market and unregulated health practices in terms of food handling and labeling practices (Cross-Contact | BeyondCeliac.org, n.d.). Furthermore, there are no definitive cures or therapies available for CD that are FDA approved, with many treatments like Ritlecitinib by Pfizer (a kinase inhibitor that targets JAK3 and TEC-family tyrosine kinases, disrupting inflammatory and immune signaling pathways also implicated in alopecia areata, that is currently being researched as a potential cure for celiac disease) and Amlitelimab by Sanofi (a fully human, non-T cell depleting monoclonal antibody that blocks OX40-Ligand, aiming to

reduce gluten-induced intestinal changes in patients with non-responsive celiac disease) still in the research and development phase (Celiac Disease Foundation, 2018). Thus, the necessity for novel treatments that address the inflammatory and intestinal homeostasis aspects of CD have never been greater.

One promising anti-inflammatory target is interleukin-10 (IL-10), a cytokine secreted by many cells such as macrophages, dendritic cells, and T cells. IL-10 inhibits pro-inflammatory cytokines like TNF- α by signaling through the JAK1-TYK2-STAT3 pathway, limiting damage to tissue and promoting immune tolerance. Most importantly, IL-10 supports the repair of the intestinal epithelial lining, an important facet in the recovery of patients with CD (Manavalan et al., 2010). Although IL-10 can exhibit pro-inflammatory effects under certain conditions, particularly in the presence of uncontrolled immune activation or in cancer microenvironments, its role in the gut in response to TNF- α is primarily anti-inflammatory and immunoregulatory. In this context, IL-10 functions to restore mucosal homeostasis and suppress excessive inflammation, making it a suitable therapeutic candidate for CD (Islam H et al. 2021).

To address the challenges of treating chronic intestinal inflammation in celiac disease, prior therapies have relied on anti-TNF- α monoclonal antibodies like infliximab and adalimumab, which block TNF- α or its receptor interactions. While these have shown success in other autoimmune diseases, they are often expensive, systemically immunosuppressive, and can have negative side effects such as carrying risks for infections and lymphoma (Hoey et al., 2019; Lee et al., 2024). *Escherichia coli* (*E. Coli*) is a safer and targeted alternative due to its delivery of therapy locally in the gut, which avoids systemic immunosuppression. As such, this research aims to engineer *E. coli* Nissle 1917 (*EcN*) to function as a localized and responsive anti-inflammatory system that triggers secretion of human IL-10 to dampen intestinal inflammation and restore immune homeostasis in patients with CD. To achieve this, *EcN* will be engineered with a plasmid incorporating a constitutively expressed Lpp-OmpA-anti-TNF- α

nanobody fusion protein under the J23119 promoter for TNF- α detection, and a PcpXP stress-response promoter controlling the expression of a YebF-IL-10 fusion protein, enabling targeted secretion of IL-10 in response to inflammatory stress.

Systems level

The proposed biological system is a living therapeutic consisting of a chassis that will function autonomously within the gastrointestinal tract of individuals with CD. This system is built around a genetically engineered chassis that will operate using a synthetic stimulus-response circuit to detect inflammatory cues and deliver a localized therapeutic response. The main goal of this system is to detect TNF- α due to its role in immune activation and mucosal damage in CD. TNF- α is the input signal for the system that will prompt a set of genetic events once it has been recognized by the chassis (Figure 1). The sensing module features a surface-anchored nanobody that binds specifically to extracellular TNF- α , which starts the signal transduction process. This nanobody, derived from camelid heavy-chain-only antibodies, is the monovalent candidate VHH#2B, selected for its high affinity, stability, and >90% sequence homology to human VH regions, reducing immunogenicity and enabling efficient TNF- α detection in the gut (Muyldermans, 2013; Part:BBa K4387996 - Parts.igem.org, 2022). Furthermore, this interaction will start the downstream genetic pathway through a promoter responsive to the TNF- α binding induced membrane stress. The physical interaction between the chassis and TNF- α via the nanobody disturbs the neutrality of the bacterial outer membrane, triggering a stress response pathway within the cell. This stress response activates specific regulatory proteins that initiate transcription from a stress-responsive promoter. This activation will lead to the transcription and translation of the human IL-10 gene which will later dampen immune responses and promote epithelial healing in the gut (Saraiva et al., 2019). To ensure effective delivery of the IL-10, the gene will be fused to a secretion tag that facilitates its export from the chassis into the external

environment, where it will be able to directly suppress inflammation, restore immune tolerance, and lessen the severity of the symptoms caused by CD. The genetic components that make up this series of events/circuit are carried on a modular plasmid that will be injected into a cell of the chassis which enables efficient construction, reproducibility, and adaptability. Additionally, this plasmid-based design will allow the genetic components and chassis to be easily modified, making it possible to reprogram the chassis to detect and respond to TNF- α if the original plan is unsuccessful or needs adjustment based on patient-specific inflammatory profiles (Brophy & Voigt, 2014).

Device level

The proposed chassis for this system is *EcN*. Isolated by Professor Alfred Nissle from Freiburg, Germany from the intestinal microflora of a young soldier who didn't develop infectious diarrhea during the Shigella outbreak in World War I, *EcN* is a gram-negative bacterium that has conventionally been used in many gastrointestinal disorders including diarrhea, uncomplicated diverticular disorder, and

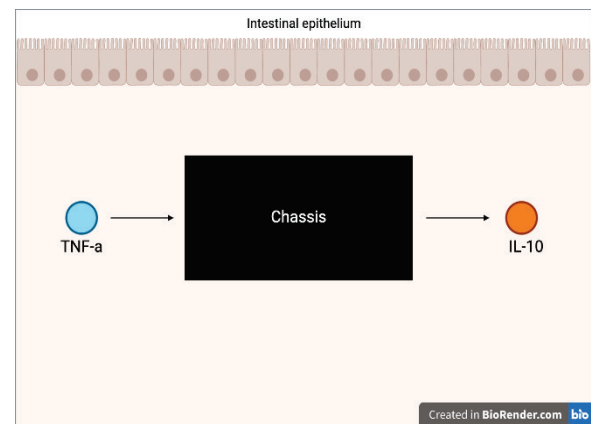


Figure 1. Schematic representation of an engineered microbial chassis designed for therapeutic intervention in the gut. Upon detection of the pro-inflammatory cytokine TNF- α in the intestinal environment, the chassis responds by producing and secreting the anti-inflammatory cytokine IL-10. This system aims to mitigate inflammation and promote intestinal immune homeostasis.

ulcerative colitis. This strain of *E. coli* lacks common virulence factors that make bacteria harmful such as α -hemolysis and P-fimbrial adhesins which makes it an optimal device. *EcN* contains microcins and multiple iron uptake systems along with adhesins and proteases which allows it to outcompete other microbes by efficiently gathering iron, adhere to the gut lining, and reinforce its ability to establish stable colonization within the gut microbiome. Additionally, *EcN* doesn't produce any known toxins, is serum sensitive, and has a non-toxic lipopolysaccharide (LPS) structure, meaning that it is easily controlled by the body and doesn't overstimulate the immune system (Scaldaferri et al., 2016). As can be seen, these characteristics make *EcN* an ideal candidate for the delivery of engineered probiotic therapies that must function safely and effectively within the human gastrointestinal tract.

The core functionality of the device is achieved through a genetically engineered *EcN* that carries a detection and response system based on TNF- α . The device is designed to detect TNF- α and respond by secreting IL-10 in order to modulate the inflammatory response associated with CD. Although studies have shown that *EcN* itself can induce gut cells to produce IL-10 within the presence of *EcN*, this approach will ensure that IL-10 is not unnecessarily produced in the absence of inflammation (Scaldaferri et al., 2016). By tightly linking cytokine detection to therapeutic response, the system minimizes the risk of immune disruption and enables precise, condition-specific delivery of IL-10 to inflamed intestinal tissue.

To enable this functionality, many key modules must be introduced into *EcNs* genetic architecture. This includes a sensing module, a signal transduction module, and response module that are detailed below.

Sensing module

As the initial component of the genetic circuit, the sensing module will be responsible for detecting TNF- α in the gut environment. In order to do this, a nanobody that specifically binds to TNF- α is expressed on the surface of *EcN*, meaning that the *EcN*

will be genetically modified to have the nanobody on its surface. The nanobody will be fused to an outer membrane protein like Lpp-OmpA, which anchors the nanobody onto the surface of the bacterium and ensures that the nanobody directly interacts with the extracellular TNF- α in the gut (Part:BBa K1991004 - Parts.igem.org, 2016). This interaction occurs when the extracellular TNF- α that is released upon the consumption of gluten binds to the nanobody, initiating the process of TNF- α detection by the *EcN*. The surface expression of the nanobody allows the *EcN* to efficiently recognize and respond to the presence of TNF- α without internalization, simplifying and fortifying the detection process of the system (Lee et al., 2024).

Signal transduction module

Upon binding of the nanobody to TNF- α , the signal transduction module becomes activated and links the TNF- α binding to downstream gene expression. This means that the molecular recognition of TNF- α by the nanobodies on the *EcN* surface will be translated into a biological response within the *EcN* chassis. The pCpxP promoter, positioned upstream of the gene encoding the anti-inflammatory cytokine IL-10, acts as a sensor of envelope or membrane stress $\rightarrow$ conditions that worsen in the inflamed gut environment characteristic of celiac disease. When TNF- α binds to the surface displayed nanobody, the interaction induces localized stress at the outer membrane, activating the CpxAR two-component system (Zhang et al., 2005). Activated CpxR then promotes transcription from the pCpxP promoter, leading to expression of IL-10. This design ensures that the binding of TNF- α triggers a tightly controlled transcriptional response, enabling the engineered *EcN* to produce and secrete IL-10 precisely in response to inflammatory signals (Saraiva et al., 2019; Hoey et al., 2019).

Response module

The final module of the circuit is the response module, and it manages the execution of therapeutic action triggered by the detection of TNF- α (Ciccocioppo et al., 2005). For our

purposes, the output is the secretion of IL-10, which will reduce inflammation in the gut through the inhibition of pro-inflammatory cytokines like TNF- α (Figure 2). Upon the activation of the signal transduction module, *EcN* expresses IL-10 coding sequence, which is then exported out of the bacterium using a secretion system like the YebF transporter protein. These secretion tags will cause the IL-10 to be released into the gut environment efficiently where it can interact with the gastrointestinal tract to reduce the effect of pro-inflammatory cytokines, alleviate CD symptoms, and most importantly, limit tissue and small intestine damage (Zhang et al., 2005).

Parts level

As mentioned previously, *EcN* will be the

chassis that will induce IL-10 production upon TNF- α detection. Specifically, the plasmid designed for this system will be engineered to include all of the components necessary to carry out the expected outcome based on the expected input. Listed below are the parts along with their specific functions within the *EcN* (Table 1 and Figure 4).

Safety

The engineered *EcN* will be constructed and tested underneath Biosafety Level 1 as it is a defined organism and unlikely to cause disease (Trapotsis, 2022). Since it is classified at this level, this means that it is suitable to be handled by people obtaining different experience levels, ranging from seasoned industry professionals to high school students. Additionally, a strain of *EcN*

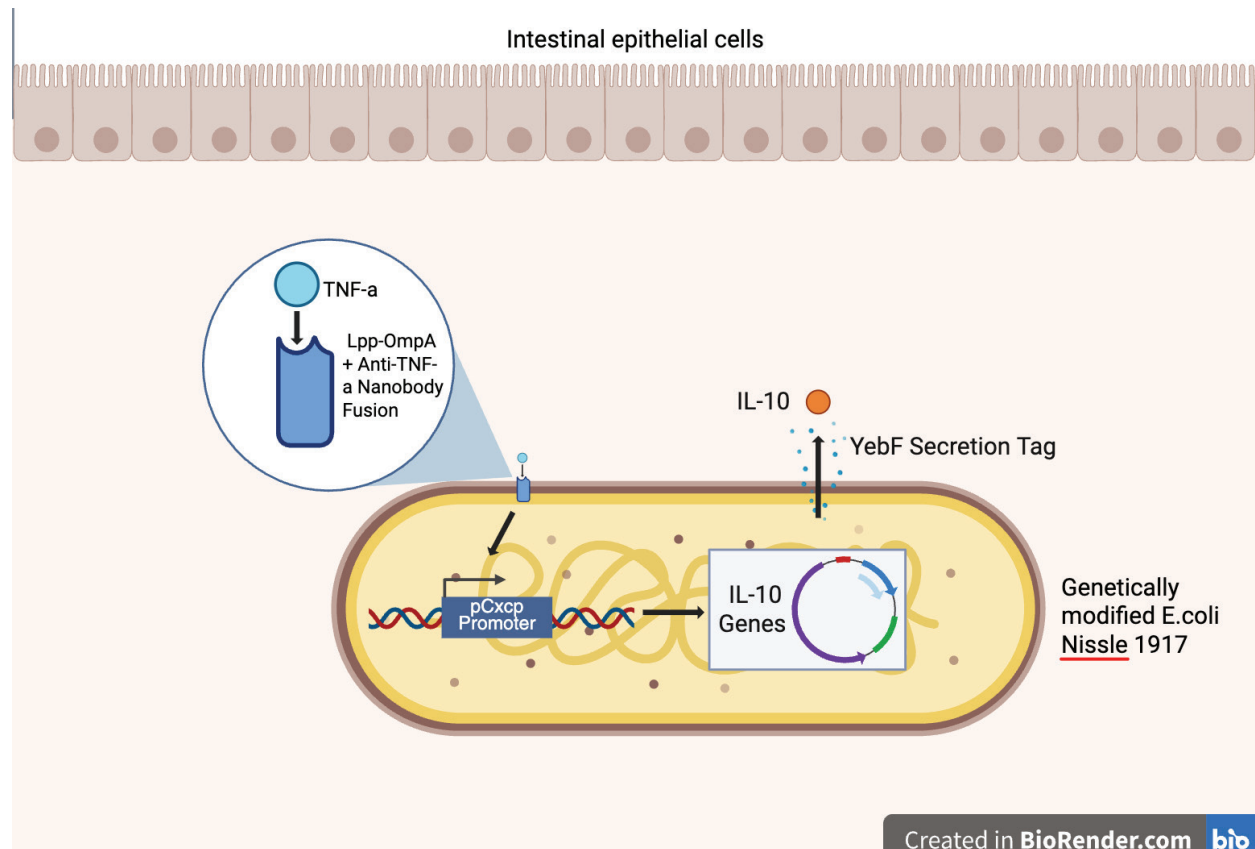


Figure 2. Schematic representation of the engineered *E. coli* Nissle 1917 therapeutic system for inflammatory regulation in celiac disease. The bacterium displays an Lpp-OmpA–anti-TNF- α nanobody fusion on its surface to detect the presence of TNF- α in the gut microenvironment. Upon binding TNF- α , the signal activates the pCxpP promoter, driving expression of IL-10 genes. The produced IL-10 is secreted via a YebF secretion tag, enabling local anti-inflammatory action near intestinal epithelial cells.

obtains the GRAS (Generally Recognized As Safe) status under FDA approval, therefore decreasing any potential risk of harm (FDA, 2023). Biosafety mechanisms like kill switches will be integrated in order to ensure that the *EcN* will be confined to the gut and not travel to an external environment outside. This means that if the *EcN* experiences a change in oxygen, pH, temperature, or is in

Table 1. A brief outline of the functional components of the engineered plasmid system in *EcN* for inflammation-responsive IL-10 secretion used in the design of the therapeutic plasmid.

Part	Function
J23119 Promoter	This promoter drives the expression of the TNF- α nanobody in <i>EcN</i> , ensuring that the nanobody is expressed at high levels on the surface of the <i>EcN</i> so that the bacterium is able to sufficiently and correctly identify TNF- α in the gut. High expression levels of the nanobody is essential for binding to the TNF- α , which allows the rest of the system to function (Part:BBa J23119 - Parts.igem.org, 2020).
Lpp-OmpA and anti TNA-a nanobody (BBa_K4387996) fusion protein	Lpp-OmpA $\rightarrow$ fusion protein that anchors the TNF- α nanobody to the outer membrane of <i>EcN</i> , enabling direct binding to extracellular TNF- α . Surface display ensures efficient cytokine detection and activation of downstream responses (Part:BBa K1991004 - Parts.igem.org, 2016). Anti TNF-a nanobody $\rightarrow$ monovalent single-domain antibody that specifically binds to human TNF- α . Its small size enables high-affinity binding, allowing precise detection of TNF- α . Upon binding, it initiates the signal transduction pathway that ultimately triggers IL-10 secretion to reduce inflammation (Part:BBa K4387996 - Parts.igem.org, 2022).
PopxP Promoter	As a stress-responsive promoter, this promoter will activate when the bacterial membrane experiences stress, which is usually under conditions like gut inflammation. Although the TNF- α binds onto the anti-TNF- α nanobody, it cannot actually activate the expression of downstream response genes including the IL-10 response gene, so this promoter will be used during the signal transduction module to perform this process. The use of this promoter will also ensure that IL-10 will be secreted only when inflammation has been detected, preventing unnecessary immune suppression when inflammation is present (Part:BBa K2026001 - Parts.igem.org, 2016).
B0034 (Ribosome Binding Site)	This ribosome binding site is placed before the coding sequences of the TNF- α nanobody and IL-10 so that an appropriate amount of ribosomes are securely bound to the mRNA sequence and begin translating the nanobody and IL-10 proteins. This ensures that the proteins involved in the detection and response phases are properly synthesized and are able to accomplish their tasks (Part:BBa B0034 - Parts.igem.org, n.d.).
YebF-IL-10	This fusion protein is important for the anti-inflammatory cytokine production aspect of the system. The YebF secretion tag enables the human IL-10 to be secreted outside of the <i>EcN</i> cell into the gut so that it can respond to the inflammation by reducing it (Part:BBa K2817009 - Parts.igem.org, 2018). Fusing the IL-10 with the YebF ensures that IL-10 is secreted only when it is needed and simplifies the transporting process (Part:BBa K554004 - Parts.igem.org, n.d.).
B0015 Terminator	This terminator is placed at the end of the plasmid to prevent the RNA polymerase from reading through the transcriptional units and mediating the gene expression to make sure that it is regulated (Shetty, 2003).
Chloramphenicol Resistance	This antibiotic resistance marker ensures that only bacteria carrying the plasmid will survive the presence of the antibiotic, Chloramphenicol (Part:BBa K2926077 - Parts.igem.org, 2019).
p15A Ori	This origin of replication is a medium-copy plasmid origin and is the site where plasmid replication is initiated. It ensures that the plasmid is correctly and efficiently replicated within the <i>EcN</i> and it is compatible with the <i>EcN</i> , which allows the plasmid to be maintained at a high copy number during bacterial cell division, maximizing the output of the system (Part:BBa K2560046 - Parts.igem.org, 2018).

an environment with a lack of gut-specific nutrients, it will self-destruct (Sterling et al., 2017). Other safety measures that can be taken include adding more triggers to the *EcN* before it secretes IL-10, such as including other pro-inflammatory cytokines or adding a limiting gene that self-destructs after a certain amount of time or number of cell replications. These steps will ensure that the production of IL-10 occurs under stricter circumstances and ensure that the treatment is temporary, controlled, and renewable. Beyond this, containment measures like confining the *EcN* in petri dishes, using sterile techniques, and proper waste decontamination protocols will be applied throughout the project to prevent bacterial escape into the environment (LaVan & Marmon, 2010). All researchers involved in the execution portion of this research will be required to obtain training in biosafety practices and follow institutional guidelines for genetic engineering/synthetic biology. This will include the personal protective equipment like lab coats, gloves, and safety goggles, the proper sanitation of laboratory areas used during the experiments, as well as a strong foundation in handling genetically modified organisms in a responsible and careful fashion. As the project progresses, continuous risk assessments and regular updates to safety protocols will be performed to account for any modifications to the organism, system design, and laboratory/industry regulations (Trapotsis, 2022). These combined practices confirm the safe development, handling, and eventual testing of the engineered *EcN*, upholding both scientific integrity and public health standards.

Discussions

The engineered *EcN* presented in this project depicts a new and focused approach to addressing the chronic intestinal inflammation associated with CD. Through the design of the modular synthetic circuit with TNF-a detecting capabilities and localized excretion of IL-10, this therapy considers a key pathological driver of CD that is often overlooked, dysregulated immune response, while decreasing the

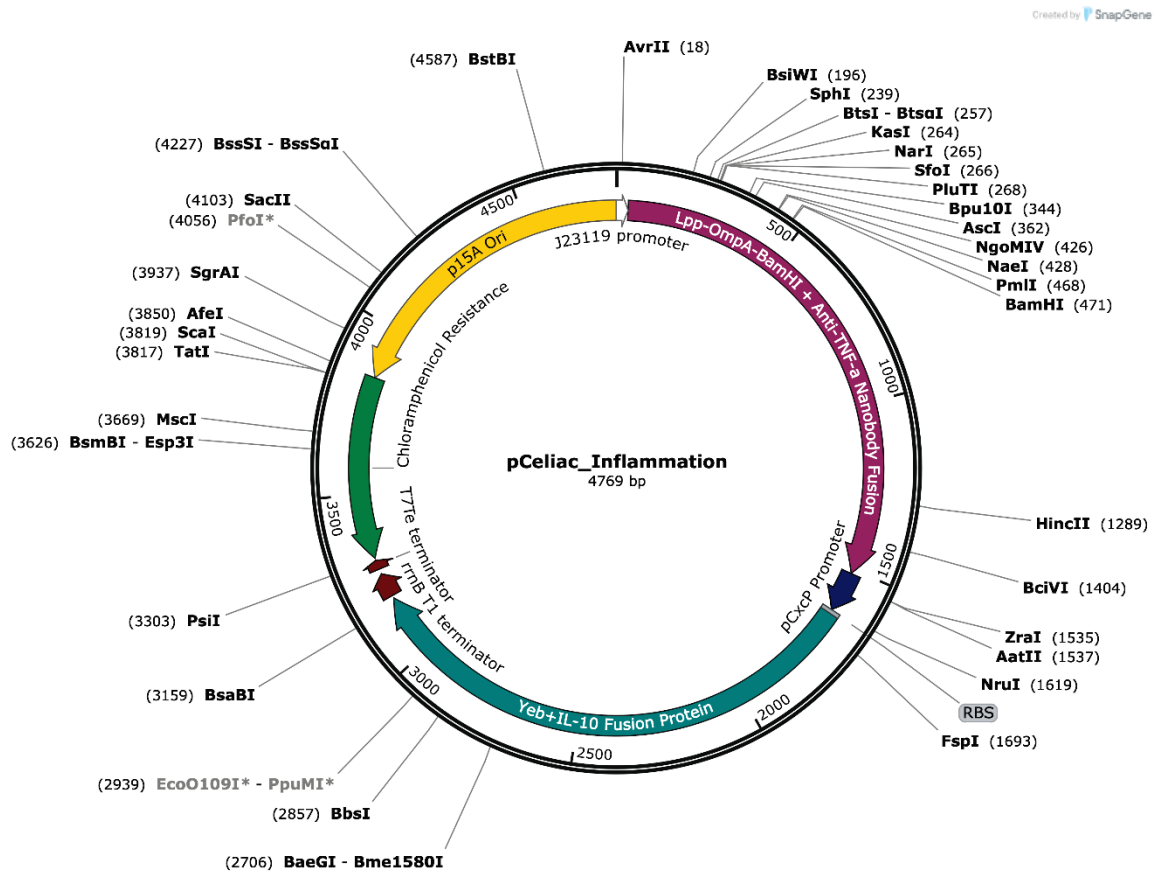


Figure 3. Engineered plasmid map encoding a TNF- α -responsive therapeutic system. This plasmid contains two expression cassettes: one featuring the constitutive J23119 promoter driving expression of a membrane-anchored Lpp-OmpA-anti-TNF- α nanobody fusion protein, and a second cassette with the inflammation-inducible PcpXP promoter driving secretion of an IL-10 anti-inflammatory cytokine fused to the YebF export signal. Standard elements include the B0034 ribosome binding site, B0015 double terminator, chloramphenicol resistance marker for bacterial selection, and the p15A origin of replication for plasmid maintenance in *E. coli*.

systemic side effects that arise in conventional treatments (Lee et al., 2024; Saraiva et al., 2019). Additionally, the specificity of the TNF- α nanobody in this genetic circuit validates that the therapeutic response is solely based on active inflammation in the gut, streamlining the system and making it conditionally responsive. Compared to current clinical approaches towards CD like lifelong gluten-free diets and immunosuppressants, this probiotic-based system offers a non-invasive, sustainable, and cost-practical alternative (Celiac Disease Foundation, 2025; Islam et al., 2021). Using *EcN* as a chassis strengthens the durability and feasibility of the system

because of its safety features, compatibility with the human intestine and gut microbiome, and ability to reproduce/clone quickly in comparison to other organisms (Scaldeferri et al., 2016; Trapotsis, 2022). Also, the IL-10 emission via the YebF lets therapeutic efforts take place without lysis of the *EcN* which preserves the functionality of the system (Jang et al., 2021). Nonetheless, several considerations remain. Since the inflammatory environment in CD patients varies in intensity and can be caused by a combination of cytokines, it may be difficult for the *EcN* system to recognize changes in TNF- α levels and significantly de-escalate inflammation (Hoey et al., 2019; Rezaei-

Tavirani et al., 2019). Differences in gut microbiome composition, pH, and host genetics might influence the impact and colonization strategy of the engineered *EcN*, causing it to display a different behavior than intended. Challenges in scaling production, consistent plasmid retention, and the prevention of horizontal gene transfer of the engineered elements to native microbes also pose potential biosafety risks (Manavalan et al., 2010; Trapotsis, 2022). Taking all of this information into consideration, future research should continue to explore multi-cytokine detection circuits, the interactions between *EcN* and the gut, and refined biocontainment strategies. If correctly implemented, this system could revolutionize the way immune-mediated gastrointestinal diseases are treated through precision, biocompatibility, and patient-centered innovation.

Next steps

To further develop the system discussed in this project, many research steps can be taken within a high school synthetic biology lab setting. The initial focus will be on constructing the plasmid that contains the nanobody-based TNF- α sensor and IL-10 secretion module and includes gene synthesis/cloning of the nanobody sequence, fusion to a suitable membrane-bound/cytoplasmic signaling domain, and the confirmation of IL-10 activation in response to TNF- α (Cameron et al., 2014; Canton et al., 2008). Once the genetic circuit has been assembled, transformation of the plasmid into *EcN* or a lab-safe strain like *E. coli* DH5 α should be performed. These experiments can include culturing the transformed cells and measuring fluorescence created from the ELISA-based detection of IL-10 in response to simulated inflammatory conditions which can be done by exposing the cells to cytokines or lipopolysaccharides (Mukherjee et al., 2021; Alper et al., 2005). The secretion system using the YebF carrier protein can also be tested by fusing IL-10 or a reporter like mCherry to YebF and assessing the presence of extracellular protein through confirmation methods like SDS-PAGE or Western blot. Safety and plasmid stability

assay should also be conducted while advancing in this project by culturing engineered strains over several generations and evaluating the output of IL-10, plasmid presence through gel electrophoresis, and statistical data. Overall, following these steps will provide basic insights into the viability and practicality of this system by using simple conditions, equipment, and readily available material to refine the design for future computational modeling, in vivo, or clinical modeling (Canton et al., 2008; Mukherjee et al., 2021).

Author contributions

SS was the sole author on this project and handled all components of it, utilizing the mentorship of Dr. Lindsey L'Ecuyer throughout the process

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